Apr 28th, 12:00 AM - 12:00 AM

Synthesis and Radical Scavenging Activity of Chromene-Hydrazone Hybrids

Andrew Ressler

Synthesis and Radical Scavenging Activity of Chromene-Hydrazone Hybrids

Andrew Ressler and Geneive E. Henry

Department of Chemistry, Susquehanna University, Selinsgrove, PA 17870

Introduction

The chromene moiety is present in a number of potent bioactive compounds possessing anticancer, antimalarial, anti-HIV, antibacterial, and antioxidant properties.[1-3] For example, calanolide A (Figure 1A) is a potent anti-HIV agent. Previous studies have shown that these properties can be enhanced or retained by modification of the chromene structure. One potential modification involves merging of the chromene core with a hydrazone functional group. Hydrazones have demonstrated antioxidant activity, as well as activity against a wide range of bacteria and fungi, exemplified by the antibacterial drug Furazolidine (Figure 1B).[4] In this study, benzenesulfonylhydrazide and 14 benzohydrazides were incorporated into chromene-hydrazone hybrid molecules, with the goal of determining the influence of structural changes on the antioxidant activity (Figure 1C).

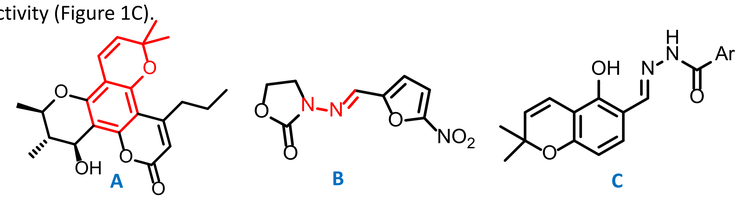


Figure 1: A) Calanolide A, with chromene in red; B) Furazolidine, with hydrazone in red; C) Chromene-hydrazone hybrid (this work)

Synthesis of Chromene-Hydrazone Hybrids

Fifteen chromene hydrazone derivatives were prepared in 2 steps (Figure 2):

STEP 1: Regioseletive chromene formation

3-Methylbut-2-enal (2 equiv) was added dropwise to a refluxing solution of 2,4-dihyroxybenzaldehyde (1 equiv) in pyridine (1 equiv), under a nitrogen atmosphere. After 24 hours, the resulting red-brown oil was concentrated *in vacuo* and purified by column chromatography to afford **1** in moderate yield (45.2%).

STEP 2: Hydrazone formation

Hydrazones **2-16** were synthesized by refluxing equimolar amounts of **1** with the corresponding hydrazide in ethanol, using acetic acid as a catalyst. The products of these reactions were purified by washing with water, followed by trituration with *tert*-butyl methyl ether, as necessary. The hydrazones were generally synthesized in good yield, ranging between 32.8% to 93.2%.

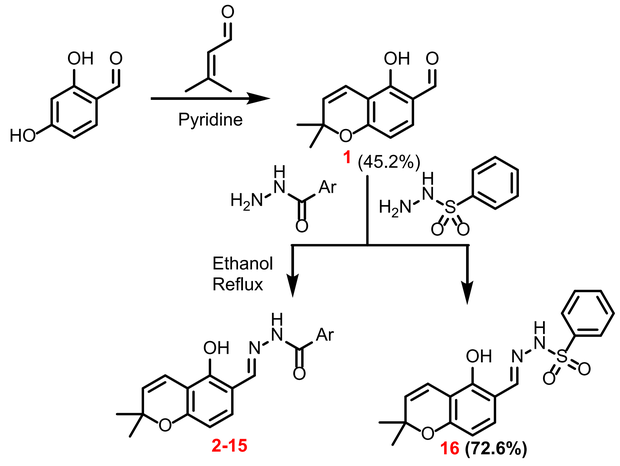
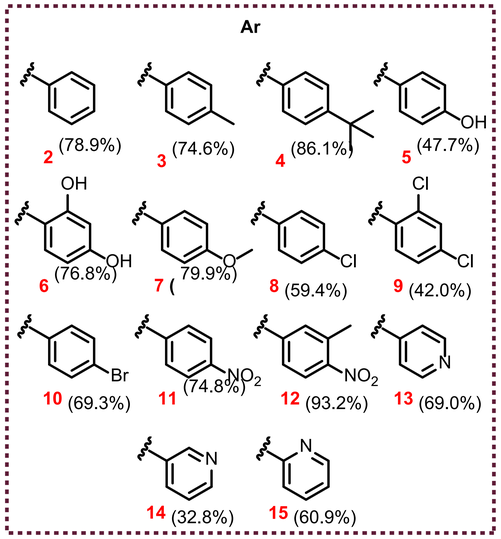


Figure 2: Synthetic route to chromene hydrazone derivatives

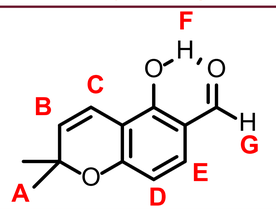


Characterization of Chromene Hydrazones

The structures of compounds **1-16** were confirmed by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. The regioselective synthesis of chromene **1** was verified by the appearance of four sets of doublets in the ^{1}H NMR spectrum, at 5.6, 6.4, 6.7, and 7.3 ppm (Table 1). The alternate regioisomer would have contained one set of doublets due to the alkene, and two singlets attributed to hydrogens in a para relationship on the aromatic ring. Intramolecular hydrogen bonding between the phenolic hydrogen and the aldehyde carbonyl was evidenced by the highly deshielded proton peak at 11.6 ppm, and the absence of a well-defined OH stretch in the IR spectrum. Successful formation of the hydrazones was confirmed by a shift in the aldehyde peak at 9.6 ppm in **1** to 8.4 ppm in the hydrazone derivatives (Table 2).

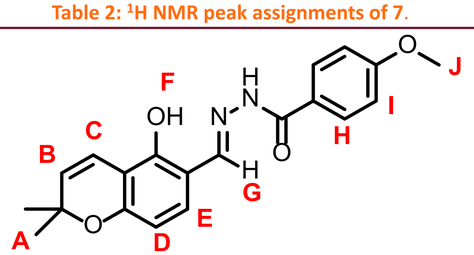
Additionally, the IR spectra of compounds **1-16** revealed that the aldehyde peak (-HC=O) present in the IR spectrum of **1** (1624 cm^{-1}) was shifted to between 1590-1605 cm^1 in the hydrazones (-HC=N-).

Table 1: ^{1}H NMR peak assignments of 1.



Position	Shift (mult.)
A	1.4 (s)
B	5.6 (d)
C	6.7 (d)
D	6.4 (d)
E	7.3 (d)
F	11.6 (s)
G	9.6 (s)

Table 2: ^{1}H NMR peak assignments of 7.



Position	Shift (mult.)
A	1.4 (s)
B	5.7 (d)
C	6.6 (d)
D	6.3 (d)
E	7.2 (d)
F	---
G	8.4 (s)
H	7.9 (d)
I	7.0 (d)
J	3.8 (s)

Radical Scavenging Activity

The antioxidant activities of the chromene hydrazones was determined using the DPPH assay to determine the influence of different substituent groups. In this assay, the stable free radical diphenylpicrylhydrazyl (DDPH) is reduced by an antioxidant, resulting in a decrease in absorbance at 515 nm, monitored by UV-Visible spectroscopy (Figure 3).

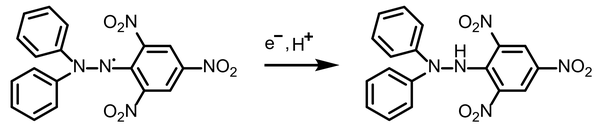


Figure 3: Diphenylpicrylhdyrazyl (DPPH) reduction reaction

Experimental Method

DPPH solution (40 µg/mL) was prepared in methanol immediately before use. Stock solutions of a positive standard, butylated hydroxyanisole (BHA), and compounds **1-16** (5 mM) were prepared in DMSO. To determine the antioxidant activity, the antioxidant stock solution (100 µL) was added to DPPH solution (3.9 mL) and incubated in the dark for 30 minutes before measuring the absorbance.

Results

Most of the hydrazone derivatives showed improved activity relative to chromene **1**, with the exception of the 2-pyridyl derivative, **15** (Table 3). Of the benzohydrazones, the dihydroxy derivative, **6,** showed the highest activity, likely due the presence of additional oxidizable functional groups. Interestingly, the presence of electron-withdrawing or electron-donating groups on the aromatic ring did not appear to affect the radical scavenging activity of the compounds. The sulfonylhydrazone, **16,** showed much higher activity than any of the benzohydrazone derivatives, with an antioxidant capacity similar to that of BHA.

Table 3: Radical scavenging activities of 1-16

Sample	% Inhibition	Sample	% Inhibition
BHA	86	**9**	6
1	4	**10**	14
2	16	**11**	16
3	16	**12**	17
4	17	**13**	15
5	14	**14**	18
6	30	**15**	3
7	16	**16**	77
8	19		

Conclusions and Future Work

In summary, 14 benzohydrazone derivatives and a sulfonylhydrazone derivative of chromene were successfully synthesized, and the antioxidant activity of each compound was tested using the DPPH assay method. Most derivatives showed low antioxidant activity relative to BHA, with the exception of the sulfonylhydrazone, which showed comparable activity.

Further studies will be conducted to determine IC_{50} values for each compound in the DPPH assay, as well as iron chelation activity using the Ferrozine assay. Furthermore, the tyrosinase inhibitory activities of the compounds will be determined using *in vitro* assays. Docking simulations and kinetic studies will be used to explore binding modes to the tyrosinse enzyme.

Acknowledgements

This work was supported by Susquehanna University and a grant from the National Science Foundation (NSF:MRI CHE 1625340).

References

1) Azizmohammadi, M. et al. 2H-chromene derivatives bearing thiazolidine-2,4-dione, rhodanine or hydantoin moieties as potential anticancer agents *Eur. J. Med. Chem.* **2013**, *59*, 15-22.
2) Fouad, S. et al. Synthesis of chromen-2-one, pyrano[3,4-c]chromene and pyridino[3,4-c]chromene derivatives as potent antimicrobial agents. *Croat. Chem. Acta.* **2018**, *91*, 99-107.
3) Tadigoppula, N. et al. Synthesis and insight into the structure−activity relationships of chalcones as antimalarial agents. *J. Med. Chem*. **2013**, *56*, 31-45.
4) Popiolek, L. Hydrazide–hydrazones as potential antimicrobial agents: overview of the literature since 2010. *Med. Chem. Res.* **2017**, *26*, 287-301.